Development of a 53BP1 transgenic Japanese medaka fish (*Oryzias latipes*) for investigation of long-term effects of low dose radiation exposure

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Emerging data from low-dose radiation studies suggest a complex organismal response in which the effects of initial radiation damage are modulated by repair and stress response pathways. Here we describe the development of a whole-animal model bearing a reporter transgene that is designed to indicate the presence of genome instability. The work is done in a genetically tractable model organism, the Japanese medaka fish. The medaka is a whole-animal vertebrate model that preserves some of the advantages of cell culture systems, including low cost, fast results, ease of gene activation and silencing, and accessibility to optical microscopy.

We hypothesized that 53BP1 (p53 Binding Protein 1) foci will be useful both as a marker for acute DNA damage and as a reporter for persistent genome instability. 53BP1 is an early participant in the DNA double-strand break (DSB) response. We created a 53BP1 fluorescent reporter transgene expressed under the control of the beta actin promoter. The expression cassette is flanked by Tol2 transposon sequences. The Tol2 system has previously been shown to increase transgenesis efficiency in zebrafish and other animal models.

We first validated the construct by introducing it into OLHNI2 medaka fibroblast cells. After 48 h, cells were irradiated with ^{137}Cs gamma rays (0.83 Gy min^{-1}) to total doses of 0, 5, and 10 Gy. Cells were fixed 30 min post-irradiation and microscope cell images collected using an Applied Precision Deltavision deconvolution microscope. A linear response for the number of foci per cell per Gy was observed for the medaka cells (~2.84 foci per Gy).

We then microinjected the construct into the 1 to 2-cell stage of T5 (semi-see through) medaka embryos. We microinjected DNA (30 ng ul^{-1}) with or without Tol2 transposase. At this time, more than 200 embryos have been injected and screened for the presence of EYFP fluorescence using an EYFP filter on a Zeiss Stereomicroscope. Embryos positive for EYFP fluorescence are being maintained at 26±1°C until adulthood is reached. To establish founder lines, EYFP positive adults will be paired for breeding with a corresponding adult wildtype T5 in order to determine germ line transmission. Results of the backcrossing will be presented.

Future experiments will investigate 53BP1 foci formation in intact medaka founder lines exposed to low doses of ionizing radiation of low-LET and HZE (high Z energetic) types. We will investigate both the acute response and the persistence of foci in animals with an early history of radiation exposure.

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